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13. ABSTRACT *(Maximum 200 words)*

The objective of this research project is to create an interdisciplinary approach in "Predictive and Alternative Toxicology," based on the integration of mechanistic toxicology and computer-based modeling systems [i.e., physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models] for the evaluation of carcinogenic activity of selected chlorobenzene isomers. The research project focuses on four of the 12 chlorobenzene isomers: 1,4-dichlorobenzene (DCB), 1,2,4,5-tettrachlorobenzene (TeCB), pentachlorobenzene (PeCB), and hexachlorobenzene (HCB). Using a variety of techniques in experimental toxicology, biochemistry, and molecular biology, a great knowledg of the roles of cell division, cell death, and mutation in relation to chemically-induced cancer is achieved. Furthermore, the development of biologically-based cancer models will reduce uncertainty in estimating the carcinogenic risk in humans from long-term exposure to chemical carcinogens and gain headway in the area of low-dose extrapolation. The long-term goal is to formulate an overall approach to predictive carcinogenic potential of chemicals where animal usage is reduced; the lengthy experimental duration is shortened; and resource requirement is minimized.

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As will be reported in the main body of the report, we have accomplished most of the originally proposed specific aims. In some areas because of the research development,we exceeded the original work scope. Although it is unrealistic to aniticipate reaching the long-term goal in the three-year project period and it was not proposed so in our porposal, nevertheless, we belive strongly that we are well on our way to achieve the long-term objective of this proposed research.

Final Report

AFOSR Grant No. F49620-94-1-0304

An Interdisciplinary and Alternative Approach to Assess the Carcinogenicity of Chlorobenzenes

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26 February, 1998

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1.0. EXECUTIVE SUMMARY

The central theme for the project was **"An Interdisciplinary and Alternative Approach to Assess the Carcinogenicity of Chlorobenzenes"** with an overall project period of three years (01/Jun/94 to 31/May/97). A no-cost extension of three months (till 31/Aug/97) was requested and approved. This request was necessary because we needed to complete experiments which were over and above the originally proposed studies [at no additional cost to the Air Force Office of Scientific Research (AFOSR)] for the purpose of completing a Ph.D. dissertation for Mr. Russell S. Thomas whose Ph.D. graduate training was mostly supported under this research grant. This final report was originally due 31/Oct/97. We were not able to complete and submit the final report until late February 1998 because: (1) We intend to append Dr. Russell S. Thomas's dissertation as part of the final report. It was not completed and submitted until the end of December 1998; and (2) Dr. Yang, the Principal Investigator (PI) of this project and the individual who was responsible for the preparation of this final report was ill with multi-level herniated discs in the lumbar region. This low back problem and its related sciatica were debilitating thereby limited Dr. Yang's ability to work.

The objective of this research project is to create an **interdisciplinary approach in "Predictive and Alternative Toxicology," based on the integration of mechanistic toxicology and computer-based modeling systems [*i.e.*, physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models] for the evaluation of carcinogenic activity of selected chlorobenzene isomers.** The research project focuses on four of the 12 chlorobenzene isomers: 1,4-dichlorobenzene (DCB), 1,2,4,5-tetrachlorobenzene (TeCB), pentachlorobenzene (PeCB), and hexachlorobenzene (HCB). Using a variety of techniques in experimental toxicology, biochemistry, and molecular biology, a greater knowledge of the roles of cell division, cell death, and mutation in relation to chemically-induced cancer is achieved. Furthermore, the development of biologically-based cancer models will reduce uncertainty in estimating the carcinogenic

risk in humans from long-term exposure to chemical carcinogens and gain headway in the area of low-dose extrapolation. The **long-term goal** is **to formulate an overall approach to predictive carcinogenic potential of chemicals where animal usage is reduced; the lengthy experimental duration is shortened; and resource requirement is minimized.** As will be reported in the main body of the report, we have accomplished most of the originally proposed specific aims. In some areas because of the research development, we exceeded the original work scope. Although it is unrealistic to anticipate reaching the long-term goal in the three-year project period and it was not proposed so in our proposal, nevertheless, we believe strongly that we are well on our way to achieve the long-term objective of this proposed research.

Using the originally revised **Specific Aims** (See **Section 3.0.**) at the time of the award as a basis, our achievement in the last three years on this project is outlined below:

- Using an 8-week *in vivo* experimental protocol (Ito's Medium Term Liver Foci assay), we obtained evidence that HCB, PeCB, and TeCB are carcinogenic while DCB is not [see Chapter 5, Ph.D. dissertation by Russell S. Thomas (Appendix 1); Gustafson *et al.,* 1998a (Appendix 2); Gustafson *et al.,* 1998b (Appendix 3); also Thomas *et al.,* 1998a,b under **Section 7.0.**].

- Using PeCB as a model chemical, we developed a PBPK/PD model which is a hybrid of deterministic and stochastic modeling. So far, we are able to use this model to simulate on computer consistently the experimental data generated in our laboratory on: (1) the pharmacokinetics of PeCB following single or multiple gavage dosing; (2) the pharmacokinetic and physiological changes in F344 rats following a 2/3 partial hepatectomy; and (3) the first two phases of the Moolgavkar-Venzon-Knudson two stage model of carcinogenesis [see Chapters 2, 3, and 6 of Ph.D. dissertation by Russell S. Thomas (Appendix 1)]. This model will serve as a prototype for the further development of an overall

predictive approach toward cancer hazard identification in the risk assessment process.

- During this project period and closely related to the modeling work carried out for this project, we developed, using PBPK/PD modeling and Monte Carlo Simulation, an approach for the assessment of the adequacy of biological exposure indices (BEIs) in humans in the occupational health and safety area. A computer program for the methodology of integrated PBPK modeling and Monte Carlo Simulation was made available for the scientific community through publication [see Thomas *et al.* 1996a,b (Appendix 4)].

It should be noted that because graduate students are involved in many of the studies in this program and one graduate student, Mr. Russell S. Thomas, used these studies as his Ph.D. dissertation, the AFOSR got much more in-depth studies (*i.e.,* exceeded original workscope) at no extra cost. Because certain experimental work, data analyses, and manuscript preparation are still on-going, we intend to issue a supplemental report at a later date to cover those areas. This additional effort will be at no extra cost to AFOSR.

2.0. INTRODUCTION

The National Toxicology Program and its predecessor, the National Cancer Institute's Carcinogenesis Bioassay Program, collectively, form probably the world's largest toxicology program (NTP, 1989). Starting in 1962, in its near 35 years operation, less than 500 chemicals have been studied for carcinogenicity and/or other chronic toxicities (NTP, 1997). These chronic toxicity/carcinogenicity studies are extremely expensive ($2-4 millions per chemical) and they require large number of animals (about 2,000 rats and mice per chemical) as well as very lengthy duration (several years per chemical) (NTP, 1997; Yang, 1997). Even though these studies are "gold standards" of the world, comparing to the approximately 600,000 chemicals in commerce (NTP, 1994), the number of chemicals with which we have adequate toxicology information for cancer risk assessment so far is minuscule. At the mode and rate of studying these chemicals as indicated above, it is doubtful that our society will ever have thorough toxicology knowledge on the majority of the chemicals that we use now or may use in the future.

It is apparent that new, alternative, less animal-intensive, shorter-term, less expensive methods must be developed if we were to have a reasonable chance to deal with the hundreds and thousands of chemicals and even more chemical mixtures in the environment.

Because of the wide scope of this research program and the tremendously large amount of data generated, we do not intend to reproduce everything in this final report. For those studies we have already published or submitted, we append copies of the reprints or manuscripts in the appendices. Since some aspects of work are still in progress, we intend to issue an supplemental report at a later date. In that supplemental report, we will also provide an update on the status of unfinished manuscripts.

3.0. OBJECTIVES AND SPECIFIC AIMS

The originally revised **Specific Aims** (per letter dated 03 January 1994) at the time of the award of the research project are as follows:

1. Evaluate carcinogenic activity of 1,4-di-, 1,2,4,5-tetra-, penta-, and hexa-chlorobenzenes in the Ito's "Medium-Term Bioassay System" using partially hepatectomized F344 rats with diethylnitrosamine as an initiator. Histopathology will be evaluated in the liver and any other tissues with gross lesions.

2. To assess time-course progression of pharmacokinetic and physiologic changes in the rat due to chlorobenzene-treatment before and after partial hepatectomy using monochlorobenzene as a model chemical for physiologically-based pharmacokinetic (PBPK) modeling studies.

3. To assess the liver cell proliferation rate (BrdUrd/immunohistochemical analysis) as a measure of hepatocellular toxicity/cell turnover in the rat following chlorobenzene-treatment before and after partial hepatectomy.

4. To assess molecular biomarkers for tumor promotion [i.e., intracellular concentration of calcium ion, protein kinase C, transforming growth factor-α (TGF-α) and transforming growth factor-β (TGF-β)] in the rat following chlorobenzene-treatment before and after partial hepatectomy.

5. Formulate an overall predictive toxicology approach for carcinogenicity in chlorobenzenes including strategy for validation studies.

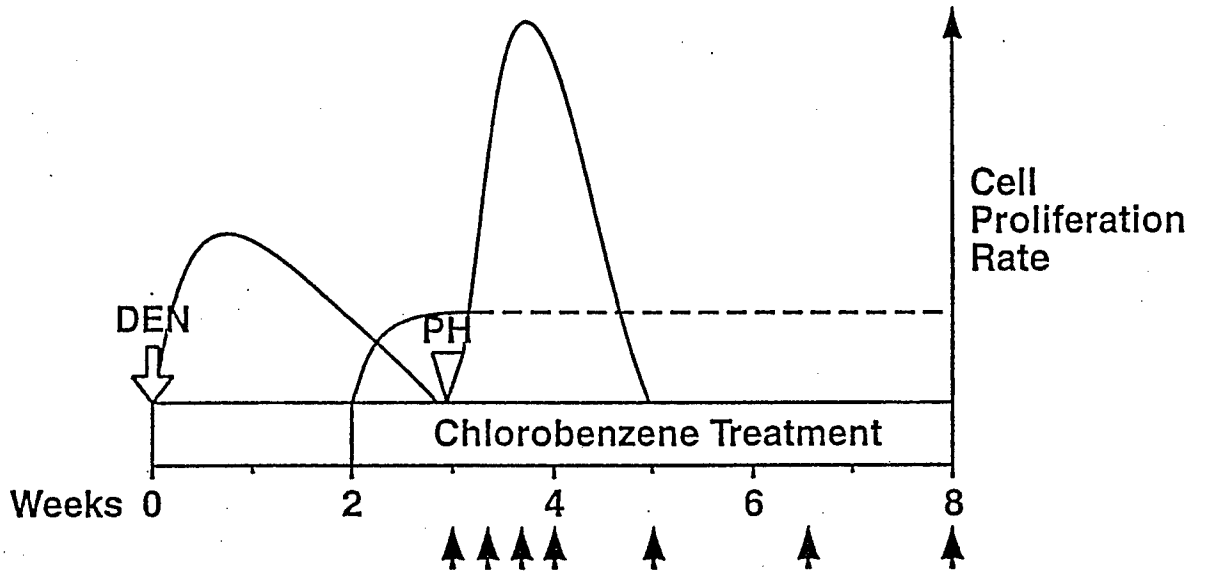
4.0. MODIFICATION OF THE ORIGINAL WORK SCOPE

It should be noted that because of the progress of the research work and experimental necessity, additional modifications on the above specific aims were made which actually resulted in more thorough work then indicated above. The relatively major modifications are listed below:

One of the most important developments of this project is related to the construction and incorporation of experimental data in the modeling of the cancer process as we studied it -- namely the utilization and the modeling of the Ito's Liver Foci Bioassay model. To achieve the above goal and as we learned more from the development of the project, we had to obtain experimental data much more in depth than what we planned originally. Since we did not have enough resources to do such in depth studies on all four isomers selected, we chose to concentrate on pentachlorobenzene. Our thinking is that we use pentachlorobenzene as a model to work out the methodology and to construct the model. The subsequent development of models for other three isomers would simply follow the method established from pentachlorobenzene studies. Even with this shift of emphasis, as demonstrated below, we actually carried out more experimentation than what we originally proposed.

1. Under Specific Aim 1 above, for each of the four isomers, we conducted <u>two</u>, instead of one, Ito's Medium Term Liver Foci Bioassay: (1) the conventional Ito's bioassay protocol as proposed originally; (2) a modified time-course Ito's bioassay as shown in Figure 1 (next page). This additional study was necessary because of the need for modeling (*i.e.,* model-directed experimentation) as well as the generation of pharmacokinetic information for the time-course events during the Ito's Liver Foci Bioassay. This represents more than doubling of effort for a major aim of the project.

2. For PBPK modeling work (see Specific Aims 2 and 5 above), instead of using



▲ = 1. Sacrifice 5 rats/group/timepoint following a 3 to 5 day BrdU exposure
2. Tissue for LI measurement
3. Tissue for molecular biology studies
4. Tissue for chlorobenzene analyses (pharmacokinetics)
5. Tissue for oxidative stress studies
6. Histopathology and morphometric analyses

Figure 1. Experimental Protocol for the Modified Time-Course Ito's Medium-Term Liver Foci Assay. (PH = partial hepatectomy; LI = labeling index)

monochlorobenzene as a model chemical for all four isomers, we chose to study the actual isomers themselves. Further, as indicated in Item 1 above, we integrated the pharmacokinetic studies with the Time-Course Ito's Medium Term Liver Foci Bioassay. This is scientifically more credible and reasonable and resulted in more demanding workload for us. Presently, we saved tissues from all the studies in -80°C freezer. Only pentachlorobenzene tissue analyses were completed for constructing the model. We intend to seek additional funds to complete the analyses of tissues from the other isomers to continue the model construction for other isomers.

3. For molecular and cellular biomarkers work (see Specific Aims 3 and 4 above), we did not do intracellular concentration of calcium ion, protein kinase C, transforming growth factor-α (TGF-α) and transforming growth factor-β (TGF-β). Instead, a variety of molecular/biochemical biomarkers related to porphyria formation and the carcinogenic process including GST-P expression, *c-jun/c-fos*, CYP1A2 expression, GSH/GSSG ratio, tissue porphyrin levels, DNA damage as measured by Fourier Transformed-Infrared Spectroscopy (FT-IR), as well as liver cell proliferation. These endpoints are deemed more relevant as biomarkers for chlorobenzene porphyria development and carcinogenesis and this modification also resulted in more work for us.

It should be noted that other relatively minor modifications in the procedures of experiments were unavoidable given the breath and depth of the research activities in this program. They are not listed individually. For details, please see Dr. Thomas' dissertation in Appendix 1.

5.0. PRESENT STATUS AND FUTURE DIRECTIONS

The future directions are presented here to indicate our overall thought process toward the eventual development of an "Alternative and Predictive Toxicology" approach for hazard identification of carcinogenic potentials of chemicals. With the experimental findings generated in this project, we are confident that we will be able to obtain other fundings to carry out further research work to reach this ultimate goal.

Ito's Medium-Term Liver Foci Bioassay

As mentioned above, two studies were completed for each of the four isomers (*i.e.,* HCB, PeCB, TeCB and DCB). Information generated have been used in hazard identification for carcinogenic potentials of these isomers in our publications. One publication is in press in *Cancer Lett.* (Gustafson *et al.*, 1998a Appendix 2) and a second one is almost ready for submission for publication (Gustafson *et al.*, 1998b Appendix 3).

Pharmacokinetic Studies

We conducted single dose, multiple dose pharmacokinetic studies on PeCB in naive rats and pharmacokinetic studies on HCB, PeCB, TeCB, and DCB as an integral part of the respective Time-Course Ito's Medium Term Liver Foci Bioassay (see Figure 1 for experimental design). All the PeCB pharmacokinetic studies and PBPK/PD modeling have been completed and they are in manuscript form [see Chapters 2, 3, 6 of Ph.D. dissertation by Russell S. Thomas (Appendix 1)]; therefore, we will be submitting at least three more manuscripts for publication in the near future. The in-life portion of pharmacokinetic studies on HCB, TeCB, and DCB as an integral part of the respective Time-Course Ito's Medium Term Liver Foci Bioassay had been completed and the tissues were saved at -80° C for future analyses. Further publications are anticipated after the completion of work on these other isomers in the future.

Molecular and Cellular Biomarkers

We collected extensive data on HCB and PeCB on the following hepatic molecular and cellular biomarkers: GST-P positive foci and related morphometric analyses, gene expressions of CYP1A2, c-*fos*, c-*jun*, GSH/GSSG ratio, tissue porphyrin levels, DNA damage as measured by FT-IR, and cell proliferation rates. For TeCB and DCB, data collected so far included GST-P positive foci and related morphometric analyses, and DNA damage as measured by FT-IR. We have tissues saved for further data collected upon securing new fundings. In addition, α-2μ-globulin binding with PeCB and a major metabolite, pentachlorophenol (PCP), was also studied due to the need of data for PBPK/PD modeling. Informaiton collected on HCB and PeCB have already been incorporated into publications [see Chapters 2, 3, 4, 5, 6 of Ph.D. dissertation by Russell S. Thomas (Appendix 1) as well as publications (Thomas *et al.* 1998a,b) listed in **Section 7.0.**].

PBPK/PD Modeling

Work on pentachlorobenzene has been completed. Using the PBPK/PD model on PeCB as a prototype, work on other isomers will be continued after obtaining additional fundings. Three manuscripts [see Chapters 2, 3, 6 of Ph.D. dissertation by Russell S. Thomas (Appendix 1)] are almost ready for submission for publication. Further publications are anticipated after the completion of work on other isomers.

6.0. RESEARCH ACCOMPLISHMENTS ON THIS GRANT

We will follow the order of the bullets in **1.0. EXECUTIVE SUMMARY** to describe our accomplishments which are directly related to the original Air Force objectives as outlined in **SECTION 3.0.**

6.1. Using an Alternative Approach for Hazard Identification of Carcinogenic Potentials of Four Chlorobenzene Isomers

Based on the Ito's Medium Term Liver Foci Bioassay, there is evidence to suggest that HCB, PeCB, and TeCB are carcinogenic while DCB is not carcinogenic. Mechanistic toxicological findings using techniques of molecular biology and bioanalytical chemistry so far have consistently substantiated our conclusions. While the demonstration of carcinogenic potential of HCB, PeCB, and TeCB experimentally in and of itself may not be too surprising to most toxicologists, this research development, under the support of AFOSR, is of great significance. In essence, the U. S. Air Force has supported a research endeavor which was able to use an alternative toxicology method (an 8-week experiment plus other supporting experiments with far fewer animals and much less resources) for carcinogenic hazard identification of four chemicals, two of which have little or no information on their carcinogenicity. Normally, under the National Toxicology Program (NTP) protocol, such an evaluation would require about 2000 animals/chemical (we use less than 200 rats/chemical), $2 million dollars/chemical (our effort so far on the entire project is less than $526,000/4 chemicals or $131,500/chemical. We say "less than" because we had done a lot of mechanistic studies for the development of predictive models.), and about 5 to 12 years/chemical (our effort on the entire project so far is about three years/4 chemicals or roughly 9 months/chemical). This is just the beginning because we intend to use the findings from this research project to further develop predictive capability for the other eight isomers in the chlorobenzene series. It is our belief that this approach (*i.e.,* PBPK/PD and cancer modeling) holds great promise in the

development of predictive and alternative toxicology for the hazard identification of chemical carcinogenesis.

6.2. Establishment of a Prototype PBPK/PD Model Which Marks the Beginning of the Development of a Predictive and Alternative Approach for the Assessment of Carcinogenic Potentials for Chlorobenzenes and Other Chemicals

Using PeCB as a model chemical, we developed a PBPK/PD model which is a hybrid of deterministic and stochastic modeling. The versatility of this model is partially reflected by the consistent computer simulations of the experimental data generated in our laboratory on: (1) the pharmacokinetics of PeCB following single or multiple gavage dosing; (2) the pharmacokinetic and physiological changes in F344 rats following a 2/3 partial hepatectomy; and (3) the first two phases of the Moolgavkar-Venzon-Knudson two stage model of carcinogenesis. Using the experience and concepts obtained in developing this PeCB PBPK/PD model, it will be relatively easy to develop similar models for HCB, TeCB, and DCB.

This marks the beginning of an approach for "Predictive and Alternative Toxicology" for the evaluation of carcinogenic potential of chemicals. Continuing development of this approach will lead to the integration of PBPK/PD and QSAR (quantitative structure activity relationship) modeling with focused mechanistically based toxicology for the prediction of carcinogenic potential of the remaining 8 isomers of the chlorobenzene series. This approach will reduce animal usage and save resources in toxicology.

6.3. Developed a Computer Program on Monte Carlo Simulation to be Coupled with PBPK/PD Modeling for the Assessment of Biological Variability

The research achievements under this and the following section are not supported exclusively by this AFOSR grant. We practice team work and much of our research concepts and ideas are developed by frequent interactions among researchers supported by a variety of grants/contracts. Thus, it is very difficult to draw clear

distinction as to which development is under what project. Much of the research effort and achievements in this and the following sections were from Dr. Russell S. Thomas while he was a graduate student under the support of AFOSR. Therefore, the AFOSR grant was undoubtedly, in part, responsible for this and the development in the following section (**Section 6.4.**).

Biologically based models with physiological parameters are becoming more popular as a tool to estimate target tissue doses from chemical exposures. However, the majority of current physiologically based pharmacokinetic (PBPK) models do not take into account the uncertainty and/or variability within the various model parameters. Consideration of uncertainty is important to evaluate the predictive ability and complexity of a model as well as identification of parameters which contribute disproportionately to variability in model output. In order to estimate the uncertainty in PBPK model output, a versatile and simple computational method was developed which can be readily incorporated into the majority of PBPK models without extensive additions to model computer code. This development was published in Thomas *et al. Fundam. Appl. Toxicol.* 31:19-28, 1996. In this paper, a separate computer program for Monte Carlo simulation is furnished. Random sample values for model parameters were included into a run-time language (command file) format which can then be utilized to execute individual PBPK models. Modifications to the PBPK model allow the desired output to be written to a data file for statistical analysis (see Thomas *et al.*, 1996a in Appendix 4).

6.4. Developed an Approach for the Assessment of the Adequacy of Biological Exposure Indices (BEIs) in Humans in the Occupational Health and Safety Area Using PBPK/PD Modeling and Monte Carlo Simulation

Protection of human health from exposure to industrial solvents is a complex problem which arises from marked inter-individual variability. We present here an approach which uses the state-of-the-art modeling techniques to provide an estimation of biological indicators based on physiology, biochemistry, toxicology, and chemical

engineering concepts. By using PBPK modeling coupled with Monte Carlo simulation, the inter-individual variability in the concentrations of chemicals in a worker's exhaled breath and urine were estimated and compared with existing biological exposure indices (BEIs). The PBPK model was constructed to simulate an exposure regimen similar to a typical work-day while exposure concentrations were set to equal the ambient occupational exposure limits (TLVs) of six industrial solvents (benzene, chloroform, carbon tetrachloride, methylene chloride, methyl chloroform, and trichloroethylene). Based on model predictions incorporating inter-individual variability, the percentage of population protected was derived using TLVs as the basis for worker protection. The results were interesting, in that the current BEIs may not necessarily protect the majority or all of the workers in an occupational setting. For instance, current end-expired air indices for benzene and methyl chloroform protect 95% and less than 10% of the worker population, respectively. Urinary metabolite concentrations for benzene, methyl chloroform, and trichloroethylene were also estimated. The current BEI recommendation for phenol metabolite concentration at the end-of-shift sampling interval was estimated to protect 68% of the worker population while trichloroacetic acid (TCAA) and trichloroethanol (TCOH) concentrations for methyl chloroform exposure were estimated to protect 54% and 97% of the worker population, respectively. Finally, the recommended concentration of TCAA in urine as a determinant of trichloroethylene exposure protects an estimated 84% of the workers. Although many of the existing BEIs addressed in this study appear to protect a majority of the worker population, an inconsistent proportion of the population is protected. As a result, the information presented in this study may provide a new approach for administrative decisions establishing BEIs and allow uniform application of biological monitoring among different chemicals (Please see Thomas *et al.*, 1996b, Appendix 4).

7.0. PUBLICATIONS DIRECTLY OR INDIRECTLY SUPPORTED BY THIS GRANT

The research work in the following papers/manuscripts was principally supported by AFOSR:

1) Thomas, R. S., Gustafson, D. L., Ramsdell, H. S., El-Masri, H. A., Benjamin, S. A., and Yang, R. S. H. 1998a. Enhanced regional expression of glutathione *S*-transferase P1-1 with co-localized AP-1 and CYP 1A2 induction in chlorobenzene-induced porphyria. *Toxicol. Appl. Pharmacol.* In Press.

2) Thomas, R. S., Gustafson, D. L., Pott, W. A., Long, M. E., Benjamin, S. A., and Yang, R. S. H. 1998b. Evidence for hepatocarcinogenic activity of pentachlorobenzene with intralubular variation in foci incidence. *Carcinogenesis.* Revised manuscript submitted for publication.

3) Gustafson, D. L., Coulson, A. L., Feng, L., Pott, W. A., Thomas, R. S., Chubb, L. S., Saghir, S. A., Benjamin, S. A., and Yang, R. S. H. 1998a. Use of a medium-term liver focus bioassay to assess the hepatocarcinogenicity of 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene. *Cancer Lett.* Accepted for publication.

4) Thomas, R. S., Gustafson, D. L., Borghoff, S. J., Long, M. E., Saghir, S. A., and Yang, R. S. H. 1998c. A physiologically-based pharmacokinetic model for pentachlorobenzene: I. Disposition following single and mutliple gavage exposures. Manuscript in preparation.

5) Thomas, R. S., Gustafson, D. L., Borghoff, S. J., Ewert, D. H., Long, M. E., and Yang, R. S. H. 1998d. A physiologically-based pharmacokinetic model for pentachlorobenzene: II. Application to a medium-term liver foci bioassay. Manuscript in preparation.

6) Thomas, R. S., Conolly, R. B., Gustafson, D. L., Long, M. E., Benjamin, S. A., and Yang, R. S. H. 1998e. A physiologically-based pharmacodynamic analysis of hepatic foci within a medium-term liver foci bioassay using pentachlorobenzene as a promotor and diethylnitrosamine as an initiator. Manuscript in preparation.

7) Gustafson, D. L., Thomas, R. S., Long, M. E., Benjamin, S. A., and Yang, R. S. H. 1998b. Comparative hepatocarcinogenicity of hexachlorobenzene, pentachlorobenzene, 1,2,4,5-tetrachlorobenzene and 1,4-dichlorobenzene: Application of molecular and cellular indices associated with a medium-term liver focus bioassay. Manuscript in preparation.

The following papers were, in part, supported by the AFOSR because some of the key authors derived their salary partially from AFOSR and the overlapping nature of the PBPK/PD modeling work and concept development:

1) Yang, R. S. H., El-Masri, H. A., Thomas, R. S., Constan, A. A. 1995. The use of physiologically based pharmacokinetic/pharmacodynamic dosimetry models for chemical mixtures. *Toxicol. Lett.* 82/83:497-504.

2) Thomas, R. S., Lytle, W. E., Keefe, T. J., Constan, A. A., Yang, R. S. H. 1996a. Incorporating Monte Carlo simulation into physiologically based pharmacokinetic models using advanced continuous simulation language (ACSL): A computational method. *Fundam. Appl. Toxicol.* 31:19-28.

3) Thomas, R. S., Bigelow, P. L., Keefe, T. J., and Yang, R. S. H. 1996a. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Amer. Ind. Hyg. Assoc. J.* 57:23-32.

4) Thomas, R. S., Yang, R. S. H., Morgan, D. G., Moorman, M. P., Kermani, H. R. S., Sloane, R. A., O'Connor, R. W., Adkins Jr., B., Gargas, M. L., and Andersen, M. E. 1996c. PBPK modeling/Monte Carlo simulation of methylene chloride kinetic changes in mice in relation to age and acute, subchronic, and chronic inhalation exposure. *Environ. Health Perspect.* 104:858-865.

5) Yang, R. S. H. 1996. Some current approaches for studying combination toxicology in chemical mixtures. *Food Chem. Toxicol.* 34:1037-1044.

6) Yang, R. S. H. (with members of the Committee to Study the Interactions of Drugs, Biologics, and Chemicals in U. S. Military Forces, Institute of Medicine, National Academy of Sciences). 1996. *Interactions of Drugs, Biologics, and Chemicals in U. S. Military Forces*, National Academy Press, 80 pp.

7) Yang, R. S. H. 1997. Toxicologic interactions of chemical mixtures, in *"Comprehensive Toxicology. Vol. 1, General Principles, Toxicokinetics, and Mechanisms of Toxicity,"* Ed. J. Bond, Elsevier Science Ltd., Oxford, England, pp. 189-203.

8) El-Masri, H. A., Reardon, K. F., and Yang, R. S. H. 1997. Integrated approaches for the analysis of toxicologic interactions of chemical mixtures. *CRC Crit. Rev. Toxicol.* 27:175-197.

9) Verhaar, H. J. M., Morroni, J. S., Reardon, K. F., Hays, S. M., Gaver, D. P., Carpenter, R. L., and Yang, R. S. H. 1997. A proposed approach to study the toxicology of complex mixtures of petroleum products: The integrated use of QSAR, lumping analysis, and PBPK/PD modeling. *Environ. Health Perspect.*

105 (Supplement 1): 179-195.

10) Yang, R. S. H. 1998. Commentary on some critical issues and concerns related to research advances on the toxicology of chemical mixtures. *Environ. Health Perspect.* Submitted for publication.

11) Yang, R. S. H., Thomas, R. S., Gustafson, D. L., Pott, W. A., Smith, T. B., Morroni, J. S., Benjamin, S. A., Verhaar, H. J. M., Hermens, J. L. M., and Mumtaz, M. M. 1998. Approaches to developing alternative and predictive toxicology based on physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) and quantitative structure-activity relationship (QSAR) modeling. Manuscript in preparation.

8.0. PRESENTATIONS DIRECTLY OR INDIRECTLY SUPPORTED BY THIS GRANT

Invited Presentations by the Principal Investigator:

1995 SOT -- EUROTOX Debate. Invited to represent SOT to debate on Chemical Mixture Toxicology Studies: For Using the Actual Mixture of Concern, ***34th Annual Meeting of Society of Toxicology,*** March 5-9, 1995, Baltimore, Maryland.

1995 Toxicology of Chemical Mixture: Overview of Environmental Pollution and Application of New Technology in Risk Assessment, ***The Conference on Environmental Toxicology,*** Academia Sinica, Taipei, Taiwan, Republic of China, May 28-31, 1995.

1995 Toxicology and Chemical Mixtures: The Real-Life Issues of Environmental Pollution and Risk Assessment, ***The Fifth Symposium on Our Environment and First Asia-Pacific Workshop on Pesticides,*** National University of Singapore, Republic of Singapore, June 5-8, 1995.

1995 The Use of Physiologically-Based Pharmacokinetic/Pharmacodynamic Dosimetry Models for Chemical Mixtures, ***International Congress of Toxicology - VII,*** Seattle, WA, July 2-6, 1995,.

1995 Biological Issues Related to Modeling Using of DCM and Other Chemicals as Examples, ***NISS/EPA/NIEHS Workshop on Statistical Issues in Mechanistic Modeling for Risk Assessment,*** Research Triangle Park, NC, September 18-19, 1995.

1995 Some Current Approaches for Studying Combination Toxicology in Chemical Mixtures, ***European Conference on Combination Toxicology,*** Veldhoven, The Netherlands, October 11-13, 1995.

1995 Toxicology of Chemical Mixtures: Issues on Experimental Approaches and Risk Assessment, ***Second Society of Environmental Toxicology and Chemistry World Congress,*** Vancouver, BC, Canada, November 5-9, 1995.

1996 Toxicology of Representative Superfund Mixtures, ***International Life Sciences Institute 1996 Annual Meeting,*** Cancun, Mexico, January 21-24, 1996.

1996 The Application of Computer Modeling to Health Effects Research, **Pacific Basin Conference on Hazardous Waste**, Kuala Lumpur, Malaysia, November 4-8, 1996.

1997 Integrating Computer Modeling and Mechanistic Toxicology for the Development of a Predictive and Alternative Toxicology Approach for Chemicals and Chemical Mixtures, **NIEHS Superfund Basic Research Program 10th Anniversary Conference on Hazardous Waste**, Chapel Hill, NC, February 24-26, 1997.

1997 Approaches to Developing Alternative and Predictive Toxicology Based on Physiologically Based Pharmacokinetic/pharmacodynamic (PBPK/PD) and Quantitative Structure-activity Relationship (QSAR) Modeling. **Colorado State University/NIEHS Conference on Current Issues on Chemical Mixtures**, Fort Collins, CO, August 11-13, 1997.

Presentations Made at Scientific Meetings:

1995

Thomas, R. S., Bigelow, P. L., Keefe, T. J., and Yang, R. S. H. 1995. Developing population based biological exposure indices: physiologically based pharmacokinetic modeling and Monte Carlo analysis. Presented at the International Congress of Toxicology - VII, Seattle, WA, July 2-6, 1995.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., and Yang, R. S. H. 1995. Developing population based biological exposure indices: Physiologically based pharmacokinetic modeling and Monte Carlo analysis. Presented at the Thirteenth Annual Meeting of the Mountain West Chapter of the Society of Toxicology. October 13-14, 1995.

Yang, R. S. H., Thomas, R. S., Moorman, M. P., and Andersen, M. E. 1995. PBPK modeling/Monte Carlo simulation of methylene chloride kinetic changes in mice in relation to aging and chronic inhalation exposure. Presented at the Annual Meeting of the Society of Toxicology, March 5-9, 1995, Baltimore, MD.

1996

Benjamin, S. A., El-Masri, H. A., Thomas, R. S., Chubb, L. S., Coulson, A. L., and Yang, R. S. H. 1996. The utilization of the medium-term liver foci bioassay for studying the carcinogenicity of hexachlorobenzene (HCB) in Fischer 344 rats. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

El-Masri, H. A., Thomas, R. S., Mumtaz, M. M., Andersen, M. E., and Yang, R. S. H. 1996. A biologically based mathematical model of the effects of partial hepatectomy on cell cycle kinetics. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

Pott, W. A., Thomas, R. S., Benjamin, S. A., and Yang, R. S. H. 1996. Morphometric evaluation of hepatocellular responses to pentachlorobenzene withing the framework of Ito's medium-term liver foci bioassay. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

Saghir, S. A., Thomas, R. S., Hwang, I. Y., El-Masri, H. A., and Yang, R. S. H. 1996. A physiologically-based pharmacokinetic model for pentachlorobenzene disposition in F344 rats. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

Thomas, R. S., Chubb, L. S., Constan, A. A., Benjamin, S. A., El-Masri, H. A., and Yang, R. S. H. 1996. A comparison of quantitative, immunohistochemical markers for cell-cycle specific changes in F344 rats. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

Thomas, R. S., Chubb, L. S., Constan, A. A., Benjamin, S. A., El-Masri, H. A., and Yang, R. S. H. 1996. A comparison of quantitative, immunohistochemical markers for cell-cycle specific changes in F344 rats. Presented at the Colorado State University Cell and Molecular Biology Spring Symposium. April 17, 1996.

Yang, R. S. H., Thomas, R. S., Chubb, L. S., Benjamin, S. A., El-Masri, H. A., Pott, W., and Coulson, A. 1996. Evidence of carcinogenic activity of pentachlorobenzene based on increased GST-P altered liver foci in F344 rats. Presented at the Annual Meeting of the Society of Toxicology, March 10-14, 1996, Anaheim, CA.

1997

Yang, R. S. H., Saghir, S. A., and Thomas, R. S. 1997. A PBPK Model for Pentachlorobenzene Disposition in Male F344 Rats Underlying Ito's Medium Term Liver Carcinogenicity Bioassay. The Toxicologists. 36:28. (Abstract)

Coulson, A. L., Saghir, S. A., Benjamin, S. A., and Yang, R. S. H. 1997. Evidence of Carcinogenic Potential of 1,2,4,5-Tetrachlorobenzene Based on GST-P Liver Foci in F344 Rats. *The Toxicologists* 36:224. (Abstract)

1998

Gustafson, D. L., Thomas, R. S., Long, M. E., Benjamin, S. A., and Yang, R. S. H. 1998. Lobular Dependent Changes in Liver Glutathione Status Following Pentachlorobenzene Treatment and Their Role in the Negative Selection of Glutathione S-Transferase π (GST π) Positive Foci. *The Toxicologists* 42:14. (Abstract)

Thomas, R. S., Gustafson, D. L., Ewert, D. H., Saghir, S. A., and Yang, R. S. H. 1998. Further Development of a Physiologically-Based Pharmacokinetic Model for Pentachlorobenzene in Male F344 Rats. *The Toxicologists.* 42:140. (Abstract)

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NTP. 1997. National Toxicology Program Management Status Report Produced From NTP Chemtrack System. 10 October 1997, p. 22.

Yang, R. S. H. 1997. Toxicologic interactions of chemical mixtures, in *"Comprehensive Toxicology. Vol. 1, General Principles, Toxicokinetics, and Mechanisms of Toxicity,"* Ed. J. Bond, Elsevier Science Ltd., Oxford, England, pp. 189-203.